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Phaeocollybia pakistanica sp. nov., the first representative of the genus from Pakistan

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Abstract—A new species of *Phaeocollybia, P. pakistanica,* is described that represents the first record of the genus from Pakistan. Diagnostic characters include purplish red to brownish red pilei, lilaceous gills, a monopodial radicating cartilaginous stipe, small ellipsoidal verruculose brown spores, and thin-walled subcapitate cheilocystidia. ITS-nrDNA sequence analyses support *P. pakistanica* as an independent new species.

Key words—*Agaricomycetes, Hymenogastraceae*, coniferous forest, pseudorhiza, Swat district

Introduction

Phaeocollybia R. Heim is a genus of agaricoid fungi, currently accepted in *Hymenogastraceae* (Matheny et al. 2006). The genus is easy to recognize in the field due to the presence of the cartilaginous stipe and deeply rooting pseudorhiza, moist to viscid umbonate pilei, and brown spores. Microscopically the genus is characterized by gelatinous tissues, roughened brown spores with an apical callus, the presence of cheilocystidia, and tibiiform diverticula (Norvell & Exeter 2008). The genus is widely distributed in forested temperate regions worldwide and currently comprises about 95 described species (http://www.indexfungorum.org/).

Species later placed in *Phaeocollybia* were first included by Fries (1838) in the heterogeneous assemblage of brown-spored agarics within the [unranked; section] *Gymnoti* under [unranked; subgenus] *Naucoria*. Kummer (1871) and Quélet (1872) independently raised *Naucoria* to generic level. Heim (1931)

established *Phaeocollybia* with *P. lugubris* (Fr.) R. Heim as type to accommodate *Naucoria* species characterized by yellow brown spores, viscid caps, and cartilaginous radicating stipes.

The genus is widely distributed throughout forests in Great Britain, Europe, Asia, Australasia, and South, Central, and North America (Norvell 1998a). Nowhere common, the genus can be considered quite rare for Pakistan, and there is no previous *Phaeocollybia* record for the country (Ahmad et al. 1997). In this paper we report the first occurrence of *Phaeocollybia* for Pakistan and describe a new species based on morphological and molecular analyses.

The specimens were collected from moist temperate forests of Malam Jabba, Khyber Pakhtunkhwa, Pakistan. Malam Jabba valley, an important biodiversity hotspot for Pakistan that hosts a variety of important medicinal and aromatic plants (Sher & Al-Yemeni 2011), lies in northern Pakistan between the Himalayas and the Hindu Kush foothills. The region (35°20′–35°45′N 72°12′–73°32′E) lies 52 km from Saidu Sharif, the capital of Swat District in the Malakand division of Khyber Pakhtunkhwa, bordering Shangla District to the northeast and District Buner to the southwest. Altitudes range from 990 m (valley entrance) to 2880 m (the highest peak of Shagar Sar; Ali et al. 2011). The valley benefits from varied agro-climatic conditions and geographic areas that facilitate a large number of important plants (Ali & Qasier 1986). The lush green coniferous forests of the Malam Jabba are also home to large numbers of important herbs, and the forest floors rich in humus and moist environments favor the growth of many macrofungi.

Materials & methods

Collection, morphological and anatomical study of basidiomata

Basidiomes were collected according to Lodge et al. (2004) slightly modified by excavation to trace their pseudorhizal origins (Norvell 1998b). Fresh specimens were photographed and important ephemeral characters and habitat data were recorded in the field. Surface features were examined using a hand lens. The collected specimens were covered in blotting paper prior to transport to the laboratory where they were dried at 40–50°C using a fan heater.

Macroscopic descriptions are based on the fresh field collections. Colors were coded according to Munsell (1975). Certain morphological and developmental terms (e.g., tibiiform diverticula, vertical-monopodia, pseudorhizae) follow Norvell (1998a,b). The dried specimens have been deposited in Swat University herbarium (SWAT) and Herbarium, University of the Punjab, Pakistan (LAH).

For microscopic examination, tissues were rehydrated in distilled water and mounted in 5% KOH, with Congo red as a stain. Anatomical features were measured using calibrated Piximètre software connected to a BOECO BM 120 compound microscope through a Byomic MVV 3000 microscopic camera and visualized on a computer screen. Twenty basidiospores, basidia, and cystidia were measured; Q = the length/width ratio

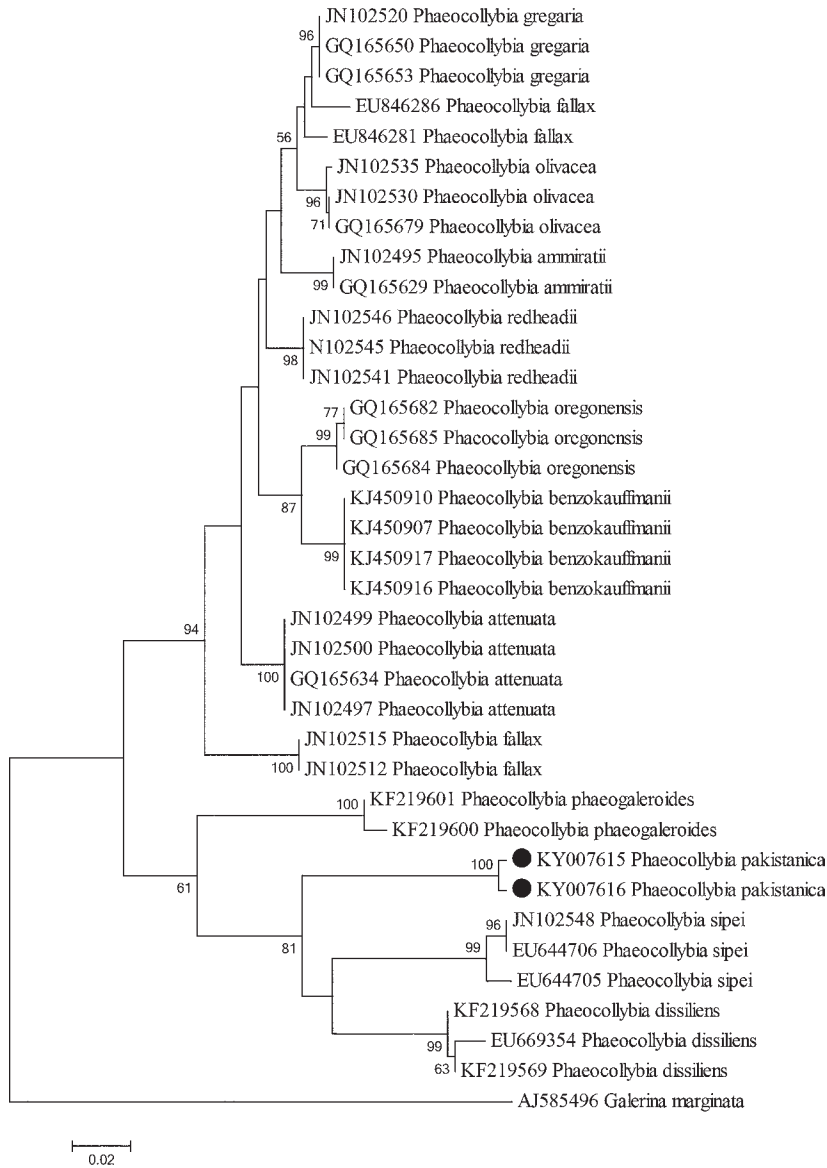

Fig. 1. Maximum Likelihood phylogeny of *Phaeocollybia* spp. Cluster support percentage is shown next to the branches. The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths representing number of substitutions per site. The analysis involved 34 nucleotide sequences. There were a total of 422 positions in the final dataset.

of a single spore; Qe = average length/width ratio of all spores; Me = average L × W of all spores measured.

DNA extraction, amplification, sequencing, and molecular phylogenetic analysis

About 50 mg of dried gills were ground into fine powder using liquid nitrogen, and the DNA was extracted according to Porebski et al. (1997). The universal primer pair ITS1F and ITS 4 (White et al. 1990, Gardes & Bruns 1993) was used to amplify the rDNA ITS (ITS1+5.8s+ITS2) region in 50 μL reaction volumes following Gardes & Bruns (1993). The PCR amplified product was sequenced by Beijing Genomic Institute (Hong Kong). BLAST analysis was performed using the National Center for Biotechnology Information (USA) database, and closely matching sequences were downloaded for further phylogenetic analysis. Sequences were aligned using MEGA 6 software (Tamura et al. 2013). *Galerina marginata* (Batsch) Kühner was selected as outgroup. Sequences are deposited in GenBank.

Results

Phylogenetic analysis — Fig. 1

Amplification of the entire ITS region using the ITSI and ITS4 primers generated a 690bp sequence. The initial BLAST comparison of the Pakistan sequences showed 85% identity and 96% query cover with *Phaeocollybia dissiliens* A.H. Sm. & Trappe (KF219569, KF219568) and 84% identity and 96% query cover with *P. sipei* A.H. Sm. (JN102548, EU644705, EU644706). The MEGA6 maximum likelihood analysis clustered our Pakistan sequences with the *P. dissiliens* and *P. sipei* sequences in a separate clade with a strong bootstrap value of 81%, thereby supporting *P. pakistanica* phylogenetically as an independent species.

Taxonomy

Phaeocollybia pakistanica J. Khan, Sher & Khalid, **sp. nov.** Figs 2, 3

MycoBank MB 818193

Differs from *Phaeocollybia sipei* by possession of clamp connections, and from both *P. dissiliens* and *P. sipei* by its lilaceous cap and gill color.

Type: Pakistan, Khyber Pakhtunkhwa Province, Swat District, Malam Jabba, 2500 m a.s.l, small groups on forest soil near *Abies pindrow* (Royle ex D. Don) Royle and *Pinus wallichiana* A.B. Jacks., 14 Sep. 2015, Junaid Khan MJ1560 (**Holotype**, SWAT15-1560; GenBank KY007615)

Etymology: referring to Pakistan, the country where the species was first collected.

Pileus 70–90 mm diam., conical to cuspidate when young, becoming convex and papillate at maturity, margin initially straight, later uplifted to revolute and undulating; edge and outer margin striate to rugulose almost two-thirds toward the central disc; viscid to glutinous when wet, shiny when dry; hygrophanous with a darker edge, color initially purplish red (10RP6), dulling to brownish red (10R7/4) and finally brownish (10R6/2) on exposure. Context concolorous with or slightly paler than pileus, thin, ≤3 mm thick at the disc and 1 mm at the

Fig. 2. *Phaeocollybia pakistanica* (holotype, SWAT15-1560). A. basidiomata in situ; B. detail of shiny pileus and violet lamellae; C. purplish-red stipe and lamellae; D. excavated basidioma showing purplish stipe apex. Scale bars = 10 mm.

outer edge, moist to dry, soft; odor fruity to mildly mushroomy; taste mild, not distinctive. Lamellae adnexed to slightly sinuate, close, color purplish with a reddish tone (7.5 RP7) at least when young before turning brown (10R4/8), edges eroded, more or less straight, crisped or curled toward the stipe in some specimens; lamellulae frequent, with crisped terminal ends, L+ll/cm ≤25. Stipe including pseudorhiza overall ≤160 mm, aerial portion <100 mm long, apex 5 mm diam.; central, terete, cylindrical or slightly widening towards the base, even slightly bulbous in some specimens; concolorous with the cap or slightly paler, with a paler purplish tinted (7.5 R8/2) upper portion and darker (7.5R6/8) lower portion without any purplish tints, glabrous or slightly fibrous when young, not bruising, hollow, cartilaginous, cortex 1-1.5 mm thick, lined with fibrils. Pseudorhiza ≤60 mm, (approximately 1/3 of overall stipe+pseudorhiza length), vertical monopodial, tapering with a blunt origin, more or less concolorous with the aerial stipe, but slightly paler orange in upper part and slightly darker below.

Basidiospores (6–)6.4–7.1(–7.2) × (3.4–)3.6–4.5 µm, Q = (1.3) 1.5–1.8 (1.9), Me = 6.7 × 4.1 µm, Qe = 1.6, ellipsoid to elongate in side view, ovoid in face view, finely verruculose in oil immersion, apiculate, brownish amber in KOH. Basidia ≤30 µm long, narrowly clavate to cylindrical, hyaline, 4-spored,

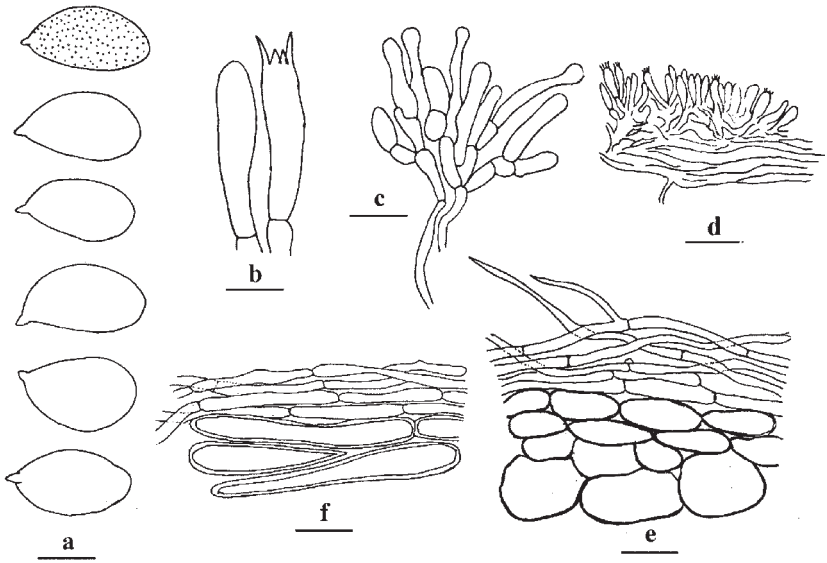

Fig. 3. *Phaeocollybia pakistanica* (holotype, SWAT15-1560). A. basidiospores; B. basidiole and basidium; C. subcapitate cheilocystidia; D. hymenium showing immature and sterigmate basidia; E. pileipellis with narrow, branched and anastomosing hyphae of the suprapellis (top) overlying shorter and wider unbranched subpellis hyphae (middle) and wider thick-walled tramal hyphae (bottom); F. pseudorhiza with rhizopellis layer shown above thick-walled tramal cells. Scale bars: A = 4.5 µm; B = 12 µm; C = 20.5 µm; D = 10 µm; E = 21 µm; F= 35 µm.

sterigmata ≤3 µm long. Cheilocystidia <40 µm long, sub-capitate, numerous, thin-walled. Pileipellis bilaminate, suprapellis a ≤23 µm-thick layer with narrow (≤2–3 µm diam) branched, gelatinized, pale brownish to hyaline hyphae with occasional clamp connections at the septa overlying a slightly darker (KOH) subpellis composed of somewhat inflated thick-walled cells (≤10 µm diam and lacking clamp connections) interspersed with ≤10 µm diam, non-septate, golden brown, branched oleifers. Pileus trama cells shorter, ≤25 µm diam., thick-walled. Stipitipellis cells ≤100 × 10 µm, cylindrical, thick-walled. Pseudorhizal pellis cells narrow, ≤5 µm diam, elongate, without any clamps. Stipe & pseudorhiza tramas sarcodimitic, with thinner branching flexuous cells intermixed among 'vessel elements' that are thick-walled, ≤250 × 30 µm, more or less fusoid, brown to golden in KOH. Clamp connections very rare; observed occasionally in pileipellis. Tibiiform diverticula present on pseudorhizal pellis.

Additional specimens examined: **PAKISTAN, Khyber Pakhtunkhwa Province,** Swat District, Malam Jabba, 2500 m asl, in small groups under *Abies pindrow*, 10 Aug

2014, Junaid Khan MJ65 (LAH35009); 31 Jul, 2016, Junaid Khan MJ1660 (SWAT16-0021)

Discussion

Molecular analysis clusters the present Pakistani collection with *P. dissiliens* and *P. sipei,* but *P. pakistanica* is both morphologically and molecularly well separated from those two species. *Phaeocollybia dissiliens* (Smith & Trappe 1972) and *P. sipei* (Smith 1957), both originally described from the North American Pacific coast, are distinguished by their bright orange to brownish orange coloration and lack the lilaceous gill colors characterizing *P. pakistanica*. The vertical monopodial pseudorhiza of *P. pakistanica* lacks the thread-like to racemose pseudorhiza of *P. dissiliens* and *P. sipei* noted by Norvell & Exeter (2008). The finely verruculose or punctate roughened ellipsoidal basidiospores are also morphologically similar to the two North American species. The rarity of clamp connections in the Pakistani collections (with a few found only in the suprapellis) further distinguishes *P. pakistanica* from *P. dissiliens* (with clamp connections relatively frequent in the pileus suprapellis and at the cheilocystidial and basidial bases) and *P. sipei* (which lacks clamp connections throughout).

Phaeocollybia spoliata E. Horak, described from India, slightly resembles *P. pakistanica* in its reddish coloration (although orange-brown more than purplish) and striate cap margin but is easily distinguished by its larger (9.5–10 × 5–5.5 µm) more heavily ornamented almond-shaped spores (Horak 1974). *Phaeocollybia rancida* E. Horak, also described from high coniferous forests in India and characterized by lilaceous violet lamellae, capitate cheilocystidia, and ellipsoid basidiospores, is easily separated by its ochraceous coloration, glutinous pilei, slightly smaller (5–6 × 3–3.5 µm) basidiospores, and absence of clamp connections (Horak 1974).

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